Plenary Session 1–Technologies

Q&A

Moderator: Greg Martin
Boyce Thompson Institute
Ithaca, New York

Dana Carroll (University of Utah): Adam, when you showed the natural variation in numbers of the TAL effectors, there were peaks at fifteen, seventeen and nineteen and a half, and valleys at sixteen and eighteen. Is that significant?

Adam Bogdanove: It's odd, but I don't know what the significance is. There are hints that it might be significant in that TAL effectors at the 5' end of the binding site often coincide with a TATA box or TATA-box like element. It could be that there is some periodicity in positioning of the activation domain for interaction—totally off the top of my head—with the transcription machinery. That's one thought.

Perry Hackett (University of Minnesota): For Dr. Ran—cells are loaded with degraded messenger RNAs, and I am wondering if they can, in collaboration with the guide RNAs that you have, lead to totally random mutagenesis inside the cell, wherein the degraded RNA might hybridize with the guide RNA in order to completely redirect it towards a non-homologous site.

Ann Ran: If I understand you correctly, your question is whether any mRNA in the cell can degrade—

Hackett: First it gets cut up by endonucleases. You have all these fragments and, before it gets completely degraded, you might have them acting as pseudo-guides.

Ran: That is a possibility. In principle, Cas9 may hijack other RNAs in the cell that have structural similarity to an sgRNA. So far, there is no good way for us to determine that directly, but we and others are trying to develop unbiased ways of detecting double-stranded breaks that might shed light on that possibility. So, we don't know yet.

Donald Weeks (University of Nebraska): First a comment on Perry's question—secondary structure of the rest of the single-guide RNA has to interact with Cas9 productively, and not any old messenger will do that. And for Ann—my question is directed towards your double nuclease cuts. I noticed that it appeared that you were getting cuts very close to each other with those two separate Cas9 single-guide RNAs. That dictates, doesn't it, that one has to hop on, make a cut, and the other has to come along and make a separate cut? Were those efficiently made? Do you see those very often?

Ran: The guides are each twenty nucleotides long, and they each recognize a twenty base-pair DNA target. So there are two Cas9 nickases that are situated close together, based on the position of the guides. The position of the guides can overlap to a certain point, but the overlap tolerance is fairly small; it can be up to about four bases and beyond that you cannot achieve double-stranded breaks. That may imply a steric hindrance limit at which Cas9 double-nicking no longer occurs, but as you pull the Cas9s farther apart, double-nicking can occur again up to a certain point; if it's longer, efficiency decreases.

Tom Turpen (Citrus Research and Development Foundation): Greg, what's the basis of the site specificity of your oligo mutagenesis?

Greg Gocal: It's homology pairing, so your oligo is homologous to the DNA sequence that you are targeting, and, generally, mismatches don't help you, although, with increased efficiency, you can push extra mismatches in terms of the DNA editing.

Turpen: So, no nuclease break is involved?

Gocal: There doesn't need to be, no.

Dan Voytas (University of Minnesota): Greg, as I understand it, the modified oligos are a mutagen—a target mutagen, but a mutagen. You showed improvements in the technology and I am wondering—now you are changing one base at a time, but I can imagine in the future changing hundreds of bases simultaneously. As the technology improves, is it your opinion that they would also have that status?

Gocal: If you're engineering a pathway that didn't exist within a crop, you may fall under an FDA or EPA regulation, despite viewing the technology as mutagenesis. In Canada, you are always going to be regulated on the basis of the trait. Speaking for myself and not Cibus, I think that this is what we will end up getting in Europe. They will regulate the trait and not the process by which the trait was developed. Time will tell. From a regulatory standpoint in Europe, we are cautious in approaching each of the individual countries and, so far, we have made progress.

Weeks: Greg, the speakers who have talked about the TALEN and CRISPR technologies have talked about off-site targeting. How mutagenic are your oligos, *vis-à-vis* other sequences in the genome? Do you have any idea?

Gocal: We have evidence from designing oligos even for targeting our model system where we put additional mismatches in or have mismatches that are displaced from the target which is generally in the center of the oligo—what we see is that as the mismatches are fifteen to twenty bases apart, you tend to disfavor those in terms of the conversion frequencies. So, at least at its basic form, additional mismatches generally push you away from the target, so then if you have an oligo of sixteen base pairs it would be relatively unique in small genomes. As you get into bigger genomes, you really have to look at what the similar sequences are. You might have several hundred members in a gene family, so the answer can be complex, related to the homology of the oligo to the target sequence you are looking at.

Joachim Schiemann (Julius Kühn Institute): Greg, did I understand you correctly that you are saying that your ODM-generated plants are not transgenic, therefore you do not follow the route for approval for genetically modified plants by approaching EFSA? You are saying okay, it's not transgenic, so you are only asking different member states. Is that right?

Gocal: A group within Europe assessed oligonucleotide mutagenesis and concluded that ODM is no different from mutagenesis. Based on that opinion, which was shared publicly, we have gone to individual member states to see if they agree with that opinion or not, in terms of being able to plant or not in those countries.

Schiemann: But the question is whether an assessment by a group of experts is legally binding. So, you think it is legally binding.

Gocal: We believe it is legally binding. The opinion of the public is a different issue.

Maria Federova (DuPont Pioneer): Canola is a global commodity. Are you looking to clarify the regulatory status with global importers of canola—not just the United States, Canada and the EU?

Gocal: We have approached the Japanese to look at that. We have approached Australia. In the decision by some countries, one of the motivators in making the decision is that our canola is now part of the global economy because we have sold it in the United States for the past several years all the time with increasing acreage. Between the regulation decision in Canada and our discussions with various groups in Europe, we feel that we are on the right track.